

REVIEW

**From Assoc. Prof. Dr. Reneta Slavova Gevrenova, Faculty of Pharmacy,
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of a PhD thesis for awarding of the educational and scientific degree “Doctor”
in Higher education area 4. Natural sciences, mathematics and informatics,
Professional field 4.2. Chemical sciences, Doctoral program ”Bioorganic chemistry, chemistry of
natural and physiologically active compounds”

Autor: Victoria Svetlinova Ivanova

**Subject: Phytochemical characterization of species of genus *Inula* growing in
Bulgaria**

Scientific Supervisor: Prof. Dr. Antoaneta Trendafilova, IOCCP, BAS

1. General presentation of the materials

Victoria Svetlinova Ivanova is an author of the abovementioned PhD thesis. She is a PhD student of independent training at the Institute of Organic Chemistry with Center of Phytochemistry (IOCCP), BAS with supervisor Prof. Dr. Antoaneta Trendafilova. Mrs. Ivanova submitted a set of materials in accordance with the Rules for the Development of the Academic Staff of IOCCP), and meets the criteria of IOCCP of acquisition of the scientific and educational degree "doctor".

Mrs. Ivanova applied a total of 6 publications in scientific journals, lists for citations and participation in conferences, and a scientometric reference according to the requirements of the IOCCP.

2. Relevance of the PhD thesis topic

Indeed, the topic of the PhD thesis is relevant due to the search for new sources of pharmaceutically important bioactive substances of natural origin for the prevention and treatment of neurodegenerative and malignant diseases. Most of these substances are secondary metabolites in plants. Asteraceae family (Sunflower family), is characterized by an exceptional variety of sesquiterpene lactones (eudesmanolides, guaianolides, germacranolides), triterpenoids, acylquinic acids (caffeyl-, feruyl-, coumaroyl-, caffeoylferuylquinic acids), methoxylated

flavonoid aglycones and glycosides. The ethnopharmacological approach of the herbal drugs is supported by the modern pharmacological achievements, focused mainly on antioxidant, enzyme inhibitory, antineoplastic and antibacterial activity. Asteraceae medicinal plants are well known for their anthelmintic, agaricidal, insecticidal and repellent activity. Sesquiterpene lactones have been extensively studied for anti-inflammatory, cytotoxic (*in vitro*), antitumor (*in vivo*), antimicrobial and antimalarial activity. In addition, caffeoylquinic acids and flavonoids are known for their antioxidant, hypoglycemic, hypocholesterolemic and antiviral properties. Their cytotoxic effect towards tumor cell lines has been demonstrated by apoptosis induction; an inhibitory activity on some membrane-bound and cytosolic proteases has also been established. In the context of the abovementioned protective effects of the Asteraceae species, it is expedient to choose the taxa of the genus *Inula* as a source of sesquiterpene lactones, caffeoylquinic acids and flavonoids. It should be noted that *I. aschersoniana* var. *aschersoniana* is a subendemic taxon for the Balkan Peninsula.

Sesquiterpene lactones and flavonoids are chemotaxonomic markers in the Asteraceae family and all identified compounds have chemotaxonomic significance for the genus *Inula*. With this respect, the choice of the secondary metabolites classes is justified and the results would be both fundamental and contributing to the chemotaxonomy of the genus.

3. Analysis and general characteristics of the PhD thesis

The PhD thesis on its goals and objectives is an interdisciplinary study, which involves competencies in the field of phytochemistry, chromatographic analysis with classical and hyphenated methods, and spectroscopic methods. The thesis consists of 117 pages and is illustrated with 37 figures, 14 tables, 156 references are cited.

The PhD student is well versed in scientific literature and expertly uses it to prove her scientific thesis.

The aim of the study is to characterize the terpenoid (sesquiterpenoids and triterpenoids) and phenolic (flavonoids and phenolic acids) profiles of three *Inula* species spread in Bulgaria: *I. britannica* L., *I. oculus-christi* L. and the subendemic species *I. aschersoniana* Janka var. *aschersoniana*, as well as assessing their antioxidant potential.

The PhD thesis consists of 3 sections: Literature survey (6 chapters), Own research (10 chapters), and Experimental part (11 chapters), together with introduction, conclusions,

contributions, bibliography and list of publications, citations and participation in scientific forums.

The Literature survey presents three key elements in the study: 1) the plant species *I. britanica*, *I. oculus-christi* and *I. aschersoniana* together with their secondary metabolites, 2) two target groups of bioactive compounds (sesquiterpene lactones and flavonoids) and 3) the general methods for isolation and identification of plant metabolites, as well as the methods for evaluation of antioxidant potential of the herbal drugs. The current data on the phytochemical composition of the assayed species are well related to their pharmacological activity.

Detailed data on the sesquiterpene lactones composition of the studied *Inula* species belonging to the eudesmanolides, guainolides, germacranolides and dimeric lactones are presented. Flavones and flavonols, including a large number of methoxylated derivatives, are also discussed in details.

A survey of two secondary metabolites classes - sesquiterpene lactones and flavonoids, which are the subject of research in the thesis, is done. The former is related to the cytotoxic (*in vitro*) and antitumor (*in vivo*) activity, while the latter is associated with antioxidant activity by the mechanism of free radical scavenging and reduction ability, diuretic and antihypertensive effect.

Emphasis is placed on the literature data related to nuclear magnetic resonance spectroscopy - the main method in the structural characterization of sesquiterpene lactones. The thesis gives detailed data on the free radical scavenging antioxidant activity (DPPH and ABTS). The literature survey convincingly substantiates the research interest in the species of the genus *Inula* and the studied secondary metabolites classes. Mrs. Ivanova analytically comments on the literature data and draws the necessary conclusions for the purposes of the work.

The Mrs. Ivanova's research is in three directions: 1) isolation and identification of secondary metabolites belonging to the sesquiterpene lactones, flavonoids, triterpenoids, sterols and phenolic acids from the studied taxa and discussion of their chemotaxonomic significance; 2) comparative qualitative and quantitative analysis of chloroform and methanol extracts of *I. britanica* originating from different localities in Bulgaria with respect to the main sesquiterpene lactones and caffeoylquinic acids and 3) Quantitative determination of phenolic compounds in *Inula* species and *I. britanica* from different localities, and assessment of their antioxidant

potential. For a brief and clear presentation of the numerous results, diagrams and figures were embedded.

In the frame of the first direction it is impressive that Mrs. Ivanova masters and successfully applies spectroscopic methods (NMR, UV, IR) and mass spectrometry, as well as chromatographic methods high-performance liquid chromatography and gas chromatography - mass spectrometry, to identify 52 compounds. In Chapter II.3 are presented important scientific results. Thirteen sesquiterpene lactones and 4 sesquiterpenoids belonging to guaianolides, pseudoguaianolides, eudesmanolides, germacranolides and 4,5-seco-guaianolides were isolated from the chloroform extracts of the assayed species. The *I. oculus-christi* study resulted in the isolation of 4 new sesquiterpene lactones (numbers 167-170) and enlarges the knowledge about the species. It should be note that the identification of 9 β , 10 β -Epoxygailardin and 9 α , 10 α -Epoxy-2-epi-gailardin is challenging due to the fact that they are isobars. *I. aschersoniana* study gave 4 new pseudoguaian sesquiterpenoids - two esters of damsinic acid (171 and 172) and aschersonianone A (173) and aschersonianone B (174). The species is a source of the known germacranolide parthenolide previously isolated from *Tanacetum* species.

The structural types of the established sesquiterpene lactones have important chemotaxonomic relevance. The Bulgarian *I. britannica* population is defined as a new chemotype being characterized with the presence of bicyclic sesquiterpene lactones (guaianolides, pseudoguaianolides and eudesmanolides). The results support the taxonomic affiliation of *I. britannica* and *I. oculus-christi* to one subgroup in the claide, based on the guaianolids and eudesmanolids found in later species. The separation of *I. aschersoniana* in another subgroup is based on germacranolides (parthenolide and diepoxycostunolide) and seco-guaianolides. The triterpenoids study in the assayed species result in the elucidation of β - and α -amyrin, taraxasterol and ψ -taraxasterol derivatives, which is a significant contribution to the Balkans subendemic *I. aschersoniana* phytochemistry (Chapter II.4). For the first time, palmitin esters of 16 β -hydroxylypeol, faradiol and 16 β -hydroxy- β -amyrin are isolated from *I. britannica*. The aforementioned compounds are also found in *I. oculus-christi*.

Flavones and flavonols are isolated from *I. aschersoniana* and *I. britannica* methanolic extracts, while *I. oculus-christi* contains only flavones (Chapter II.5). In line with the flavonoid composition of the tribe Inuleae, C-6 methoxylated derivatives jaceosidine, hispidulin, hispidulin 7-glucoside, patulitin, patulitrin, patuletin-7-*O*-(6"-*O*-acetyl)- β -D-glucopyranoside are identified.

For the first time jaceosidine was reported in the genus *Inula*; patuletin-7-O-(6"-acetyl) glucopyranoside is a newly discovered natural compound.

One monocaffeoylquinic acid (chlorogenic) and two dicaffeoylquinic acids (1,5- and 3,5-dicaffeoylquinic acid) are isolated from the of *I. oculus-christi* and *I. britannica* methanolic extracts. 3,4- and 4,5-dicaffeoylquinic acid are evidenced by high-performance thin layer chromatography (HPTLC) and comparison with reference standards. Although acylquinic acids are well known major compounds in the Asteraceae family, they appropriately complement metabolic profiles in this study.

I highly appreciate the investigation of the Bulgarian *I. britannica* populations for qualitative and quantitative determination of sesquiterpene lactones gailardin and britanin, and caffeoylquinic acids by HPTLC, and GC and HPLC, respectively, with subsequent hierarchical clustering. The results show that Bulgarian populations from the regions of Berkovitsa and Slavyanka have a high content of caffeoylquinic acids, while this one from the village Uglen contains the greatest quantities gailardin and gritanin.

Comparative analyses of the total phenolic and flavonoid content in chloroform and methanol extracts of the assayed species show that the *I. aschersoniana* flowers have the highest quantities from both classes. A good correlation was observed between total phenolic and flavonoid content of the methanol and chloroform extracts from leaves and flowers of the three studied taxa and their ability to scavenge free DPPH and ABTS radicals. In the same way, the highest antioxidant activity of *I. britannica* populations originating from Berkovitsa and Slavyanka is consistent with the high content of total phenols and flavonoids registered in the extracts.

The Mrs. Ivanova's thesis makes an impression with the mastery level and appropriate application of a number of spectroscopic and chromatographic methods. The results are statistically reliable and hypotheses have been made for possible practical application.

4. Characteristic of scientific contributions

The thesis contributions are in the field of phytochemistry and chemotaxonomy, as well as in a modern direction of the plant extracts research - prioritization of the extracts at an early stage of research based on the integration of metabolic profiling with biological potential tests. Indeed, the isolation of 9 new natural compounds is a fundamental contribution – among them, 4 sesquiterpene lactones, 4 pseudoguaian sesquiterpenoids and 1 flavonoid acylglucoside.

The chemosystematics of the genus *Inula* is updated with a total of 52 natural compounds - sesquiterpenoids, triterpenoids and sterols, flavonoids and phenolic acids. A new chemotype of *I. britannica* producing bicyclic sesquiterpene lactones has been established.

Promising Bulgarian populations, rich in valuable sesquiterpene lactones britannin and gaylardin, as well as caffeoylquinic acids chlorogenic, 3,5-, 1,5-, 4,5- and 3,4- dicaffeoylquinic acids, were selected among 11 studied ones. On the other hand, the antioxidant potential of the leaves and flowers extracts from of *I. britannica*, *I. oculus-christi* and *I. aschersoniana* var. *aschersoniana* by the mechanism of radical scavenging was evaluated together with *I. britannica* extracts originating from 11 Bulgarian localities. Very good correlation was established between the total phenolic and flavonoid contents and the radical scavenging activities of the samples was obtained.

5. Publications

The results of the PhD thesis are published in 6 publications, one of which with Q1 and three - with Q2, and are presented at 7 scientific forums. Very good impression is made by the fact that 4 of the publications have 16 citations.

The research is funded in 3 scientific projects: 1) PhytoBalk (supervisor Assoc. Prof. Dr. K. Danova) - The Bulgarian-Swiss Research Program and the Ministry of Education and Science, 2) project DN09/11 (supervisor Prof. Dr. A. Trendafilova) funded by the Research Fund, the Ministry of Education and Science and 3) The National Research Program “Young Scientists and Doctoral Students” (DCM 577/17/08/2018) of the Ministry of Education and Science.

6. Critical remarks

I have no critical remarks on the hypothesis and design of the research and the PhD itself. I recommend continuing and expanding the study with other species of the genus *Inula* and Asteraceae species.

I highly appreciate the Mrs. Ivanova’s work in the field of phytochemistry and I believe that the results of her PhD have an original scientific and applied nature.

Conclusions

The presented PhD thesis has original scientific contributions in the field of phytochemistry and chemotaxonomy. Mrs. V. Ivanova masters spectroscopic and chromatographic methods and achieved fundamental and applied results. The thesis meets all the

requirements of Law for the Academic Staff Development of the Republic of Bulgaria and the Rules for the implementation of the Law. I find it appropriate to give my positive assessment and to recommend to the Scientific Jury to award Ms. Victoria Svetlinova Ivanova the educational and scientific degree "Doctor".

14.06.2022

Reviewer:

(Assoc. Prof. Dr. R. Gevrenova)